

Related Species of *Trididemnum* in Symbiosis with Cyanophyta

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KOTT, P. Related species of *Trididemnum* in symbiosis with Cyanophyta. *Proc. Linn. Soc. N.S.W.* 107 (4), (1983) 1984: 515-520.

A new species of *Trididemnum* from the Great Barrier Reef in apparently obligate symbiosis with Cyanophyta is described. It appears to be closely related to *T. clinides* Kott, known from the same habitat. Differences in colony form, in the size, shape and distribution of spicules and in the size of the zooids and larvae, distinguish the species from one another.

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INTRODUCTION

Ten of the 20 didemnid species previously known to be associated with prokaryotic plant cells are contained in the genus *Trididemnum* (Kott, 1982). Four species of *Lissoclinum*, 3 species of *Diplosoma*, 2 species of *Didemnum* and one species of *Echinoclinum* are known to have similar symbionts (see Kott, 1982). The large number of *Trididemnum* spp. with associated plant cells is more surprising in view of the relatively small number of *Trididemnum* spp. in the tropical environment when compared with the diversity of the genus *Didemnum* (see Kott, 1981). The symbiosis is most often obligatory, although non-obligate symbionts occur in patches on the surface of some species (Kott *et al.*, 1984).

In the majority of cases the plant cells involved in symbiosis with *Trididemnum* are *Prochloron*, although Cyanophyta are also known to occur (see Kott, 1982).

Two closely related species of *Trididemnum* with obligate prokaryotic symbionts are discussed below and compared with one another. One, a new species described for the first time, has only Cyanophyta in the test. The other, *T. clinides* Kott, 1977, recorded previously from a wide geographic range, has both Cyanophyta and *Prochloron* (*pers. comm.* G. C. Cox). It is redescribed in order to clarify its relationship to the new species. These two related species differ from most other small algae-bearing species of this genus in having an atrial siphon rather than a sessile opening. *In situ* the species look almost identical and occupy the same habitat on weed and under rubble near the low tide mark, behind the reef crest. Their symbionts are embedded in the test as in the equally small species *T. miniatum* Kott, 1977, that is also recorded from similar habitats in the Great Barrier Reef. *Trididemnum miniatum* is readily distinguished by its small spherical spicules and sessile atrial aperture.

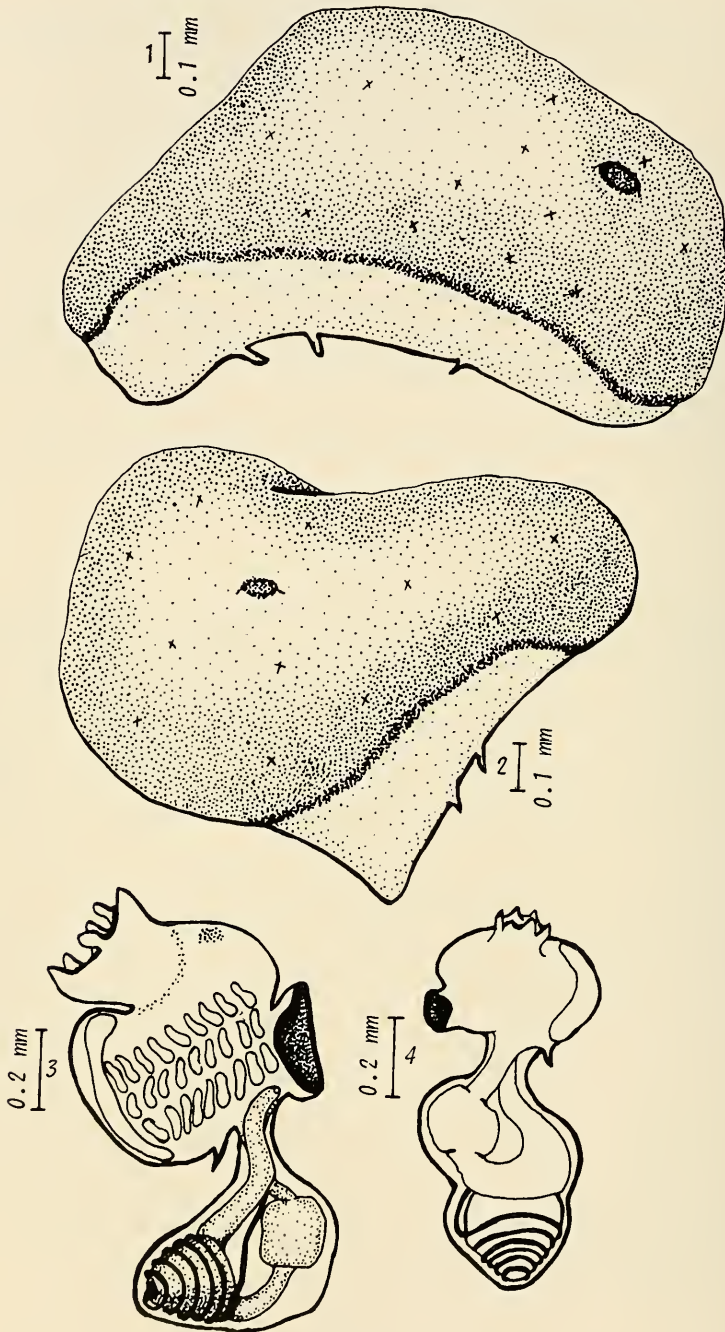
Types of both species are located in the Queensland Museum (QM).

***Trididemnum tegulum* n.sp.**

Figs 1-3; 5

DISTRIBUTION

Records: Heron I. low tide under boulders, on weed and rubble. Holotype QM GH1492. Paratypes, QM GH892, GH1337, GH1350, GH1493, GH1494, GH1495.



Figs 1-4. 1, 2. Colonies of *Trididemnum tegulum* n. sp. 3. Zooid of *Trididemnum tegulum*. 4. Zooid of *Trididemnum clinides*.

DESCRIPTION

Colony: The colonies are small, firm, almost cartilaginous cushions up to 10 mm in greatest dimension and up to 6 mm thick. The upper surface is almost hemispherical and the diameter narrows to the sometimes pointed base. Larger colonies are elongate and appear to lobulate. Zooids are arranged in a single or at most two circles around the outside of the upper rounded surface. There is a large, but only slightly protruberant common cloacal opening in the centre of the upper surface, or in elongate colonies 2 to 3 common cloacal openings are present, equidistant from one another along the length.

In the living specimen, the highly-arched surface is brownish-black. This gradually fades to white about half way down the sides of the colony.

The test is firm but easily cut. There is a superficial layer of bladder cells all around the upper surface, the sides and base of the colony. On the upper half of the colony, spicules are moderately crowded to sparse in a thin layer beneath the superficial bladder cell layer. In the thick basal test beneath the abdominal common cloaca they are always rather evenly spaced and are not crowded into a layer beneath the bladder cells either around the borders or base of the colony. Occasionally spicules are also present at abdominal level. However they are very sparse, or absent altogether from the fleshy test at thoracic level. Clumps of spicules are present in the test around the branchial apertures and these are the only spicules that protrude through the bladder cell layer. There is a small group of spicules where each atrial aperture opens into the common cloacal cavity. Prokaryotic plant cell symbionts, 0.01-0.015 mm diameter, are embedded in the bladder cell layer and throughout the test at the level of the thoraces, although they become progressively less crowded away from the surface. They are not present beneath the cloacal cavity. Minute dark rounded or oval cells about 0.005 mm in diameter are present throughout the upper layer of test (beneath the bladder cells and lining the cloacal cavity) and in large oval reservoirs in the basal test. This dark pigment, scattered through the deep thoracic layer of the test is the cause of the black opaque pigmentation of the upper half of the colony, while the lower half is off-white, owing to the embedded spicules and the absence of dark pigment cells. The dark pigment usually masks the symbionts, unless they are especially numerous in the upper layer of test.

In preservative, the colour of the symbionts fades more rapidly than the black of the ascidian cells and the upper half of the colonies remain grey-black, only a slightly lighter shade than they are in their natural habitat. Occasionally small spherical or rod-shaped pink cells (0.007-0.009 mm) are present in the test amongst the green symbionts.

In the smaller colonies the central test is uninterrupted from the base of the colony to just beneath the cloacal aperture where it sometimes forms a plug projecting up into the aperture. The cloacal cavity slopes deep into the colony around this central core and breaks into canals around the zooids at abdominal level. The firm uninterrupted central core of test, and thick surface layer around the thoraces in the periphery of the colony maintain the conspicuously arched contour of the upper surface of these colonies. In elongate colonies, before lobulation, there is a shallow cloacal cavity that extends their whole length and slopes off into the abdominal canals around the sides.

The spicules are 0.02-0.05 mm in diameter. They are very variable. More than half are of the usual stellate form with about 9 conical rays in optical transverse section. However there are also burr-like spicules with very fine needle-like rays and others with very numerous blunt and/or pointed rays.

Zooid: Zooids are just over 1 mm long. The branchial siphon is large with 6 very conspicuous lobes. The atrial aperture is on a short and conspicuous funnel-shaped

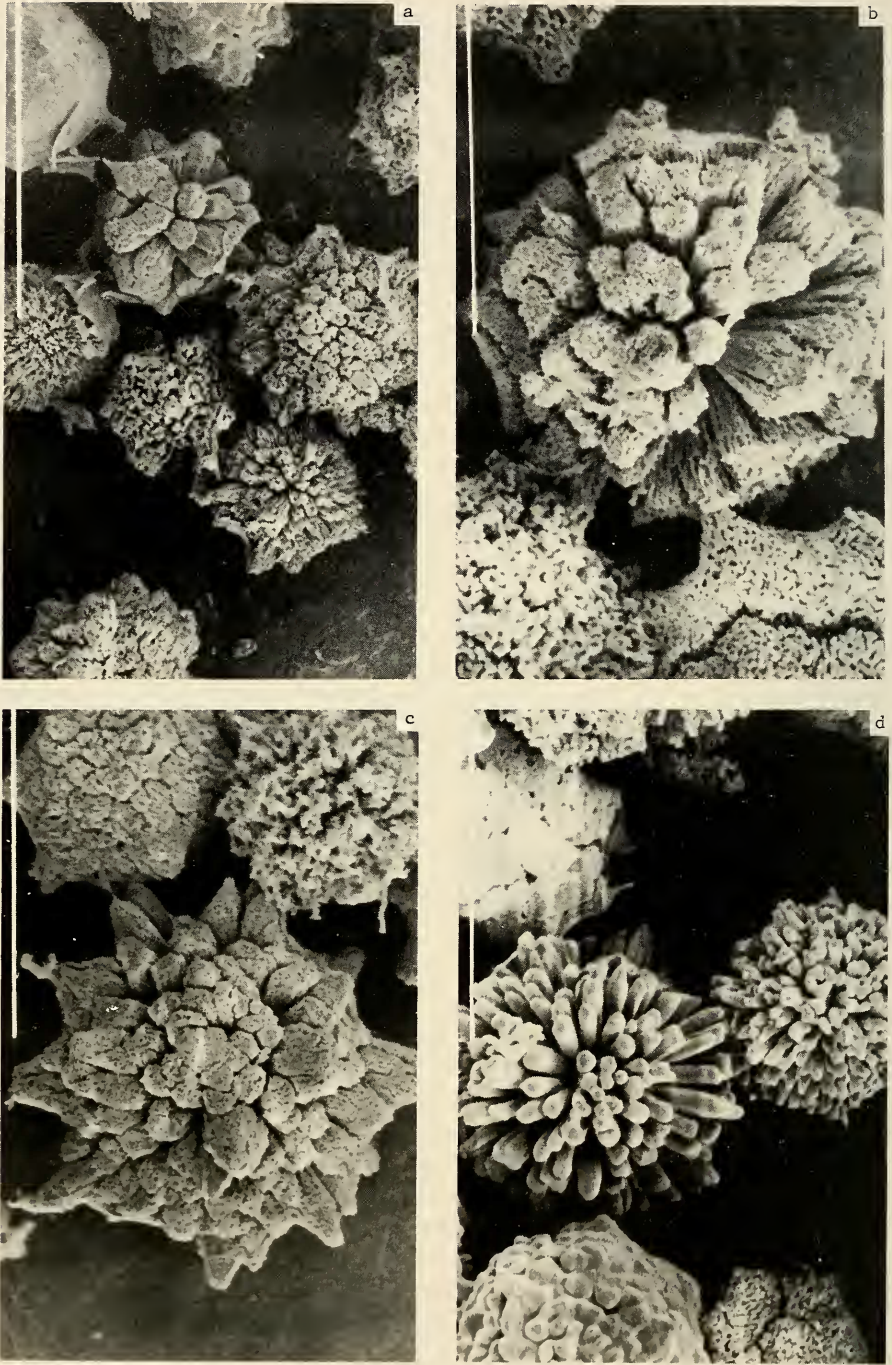


Fig. 5. Spicules of *Trididemnum tegulum* (small scale line = 0.0005 mm).

siphon from the posterior third of the dorsal surface. There is a long prebranchial area and 3 rows of 9 long, oval stigmata. The thorax is over half the length of the body. There are fine longitudinal thoracic muscles, but the retractor muscle is very small, projecting only slightly from the thorax, at the base of the endostyle.

The stomach is small and yellow and the gut forms a rounded loop, flexed ventrally. The ♂ follicle on the dorsal or under side of the gut loop is pointed and there are 5.5 coils of the vas deferens.

Larva: The numerous colonies collected in January 1982, 1983 and August 1982 had no embryos, and only a single larva was present in a colony collected in June 1982. The trunk is 1 mm long and is completely enveloped in symbiotic algal cells, these being absent only from a small area above the larval sense organs. The three adhesive organs also protrude through the plant cells.

Remarks: The species is distinguished from *T. clinides* by its highly-arched upper surface, large zooids and larvae, abdominal cloacal canal, and by its larger and more variable spicules and their distribution, being found principally in the lower half of the fleshy colonies. The thick surface layer of test that extends the full length of the thoraces has dark pigment that is not obscured by the spicules which are principally confined to the basal layer of test beneath the common cloacal cavity and cause only the lower half of the colony to be off-white. This is apparent from the outside of the thick colony, contrasting with the brown-black pigment in the zooid-bearing upper part.

***Trididemnum clinides* Kott, 1977.**

Fig. 4

Trididemnum clinides Kott, 1977: 671; 1980: 5 and synonymy; 1982: 109.

DISTRIBUTION

Records: Philippines, Eniwetok, Guam, Heron I.

DESCRIPTION

Colony: The colonies are small (up to 5 mm in diameter and not more than 3 mm thick). Preserved specimens are usually flat on the upper surface, the border sometimes slightly raised. The surface contour of the preserved specimens is a result of the relatively thin layer of surface test that is depressed over the thoracic cloacal cavity, while the borders of the colony are supported by the crowded layer of spicules that usually occurs just beneath the bladder cell layer around the borders of the colony. The surface layer of test, limited by the position of the cloacal cavity, is relatively thin and has a thin bladder cell layer and a thin layer of spicules, that are often very sparse except over the anterior end of the zooids where these open to the surface. The basal layer of test, beneath the cloacal cavity, is thick and contains the embedded abdomens and moderate to sparsely distributed calcareous spicules. The spicules are evenly distributed except around the base and borders of the colony, where they are crowded together beneath the thin bladder cell layer. They are also present in small groups where the atrial aperture opens into the cloacal cavity. Spicules are 0.03-0.04 mm in diameter with 9-11 rounded or conical rays. Prokaryotic symbiotic algae are present in the surface and basal layer of test, very much reduced in numbers beneath the cloacal cavity. Eukaryotic (Chlorophyta) green algae are also usually present in the test.

Zooid: The zooids are arranged around the periphery of the colony, opening onto the upper surface around a central cloacal aperture. They are never more than 1 mm long, usually less, and their small size is emphasized by the fact that the thorax is always contracted in fixed specimens. There are 5-6 stigmata in each row. The thorax is always smaller than that of *T. tegulum*. The retractor muscle is minute, as in *Trididemnum tegulum*. In both species the branchial siphon is large and has conspicuous pointed lobes. The abdomen does not differ appreciably from that of *T. tegulum*.

Larva: Larvae are small, the trunk being 0.6 mm long. They otherwise resemble larvae of *T. tegulum* with a complete envelope of algal cells exposing only the larval sense and adhesive organs. It is not known if the symbiotic cells that surround the larval trunk are *Prochloron* or Cyanophyta or both.

Remarks: In this species the greater part of the thickness of the colony is beneath the shallow thoracic cloacal cavity. The spicules present in this basal layer of test are especially crowded superficially and create the bright white border and base of the colony by which this species can be readily distinguished from *T. tegulum*.

Since analysis of pigments is based on whole colony extracts, the presence of *Prochloron* is masked by the Chlorophyta which usually occur in the test of *T. clinides* (see Kott, 1982). The presence of Chlorophyll a and b and phycobilin pigments indicates the presence of Cyanophyta and of *Prochloron* and/or Chlorophyta. The presence of *Prochloron*, in addition to the Chlorophyta known to occur, must be determined by studies on the morphology of the prokaryotic cells present.

ACKNOWLEDGEMENTS

I am grateful to Guy C. Cox (University of Sydney) for information on the identity of the symbionts. David Parry (University of Queensland) collected many of the specimens on which this work is based and determined the pigments present. G. C. Cox and A. Larkum of Sydney University also supplied specimens.

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